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Hidden in the bamboo: A new parachuting frog (Rhacophoridae, *Rhacophorus*) from the borderlands of western China, with comments on the taxonomy of *R. rhodopus*

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Abstract

The Gaoligong Mountains are characterized by large variations in elevation and topography, which support high levels of biodiversity and endemism that remain largely understudied. Herein, based on the integration of morphological comparisons and phylogenetic reconstruction, we describe a new species of *Rhacophorus* from the northern Gaoligong Mountains, Yunnan Province, China. The new species, *Rhacophorus dulongensis* **sp. nov.**, is morphologically distinguishable from its congeners based on the differences in body size, head length, tibia length, snout and tongue shape, toe webbing formula and coloration, ventral skin texture and coloration, dorsal pattern and coloration, body macroglands, iris coloration, and pattern of markings on flanks. Phylogenetically, it differs from its congeners by uncorrected *p*-distances of >4.8% for the 16S rRNA gene fragment. *Rhacophorus dulongensis* **sp. nov.** is likely to be found in Myanmar, considering its type locality lies close to the China-Myanmar border. The phylogenetic analysis revealed that the "widespread" species, *R. rhodopus*, is a species complex and a composite of five distinct lineages. The results revealed that *R. napoensis* is also found in Vietnam, making it a new country record for Vietnam. Interestingly, *R. dulongensis* **sp. nov.** likely breeds in bamboo, a hidden behavioral characteristic that makes them easy to overlook. Given the ongoing habitat loss and degradation in the region, further biological exploration is urgently needed in the Gaoligong Mountains as a biodiversity reservoir.

Key Words

Biodiversity hotspot, frog, Gaoligong Mountains, new record species, new species, systematics

* These authors contributed equally to this work.

Introduction

The Gaoligong Mountains, situated longitudinally along the border of China and Myanmar, are at the convergence of three key biodiversity hotspots: Indo-Burma, the Himalaya, and the Mountains of Southwest China (Myers et al. 2000). These mountains extend north-south in the western part of Yunnan Province, China, and are drained by the Irrawaddy River on the west and the Salween River on the east (Chaplin 2005). Due to its large variation in elevation, multiple latitudinal belts, and complex topography, which provided a wide range of neighboring climatic and structural niches, this region represents a classic example of a montane hotspot for biodiversity and endemicity (Ricketts et al. 2005). Recently, the rate of new species discovery from the Gaoligong Mountains has been high, including new primates (e.g., Geissmann et al. 2011; Fan et al. 2017; Hu et al. 2022; Li et al. 2024). Despite this, amphibians and reptiles in the northern Gaoligong Mountains have remained poorly studied because of difficulties in accessing the region previously (Liu et al. 2021; Wu et al. 2021), suggesting that our knowledge of the region's herpetofaunal diversity is incomplete.

Currently, the frog genus *Rhacophorus* Kuhl and Van Hasselt, 1822, contains 43 species, distributed widely across Asia, including India, Myanmar, Bangladesh, Laos, Thailand, Vietnam, Cambodia, Malaysia, Brunei, and Indonesia, as well as southern China (Jiang et al. 2019; Frost 2024). At present, the following eight species of *Rhacophorus* have been recorded from China: *R. bipunctatus* Ahl, 1927; *R. kio* Ohler & Delorme, 2006; *R. laoshan* Mo, Jiang, Xie, & Ohler, 2008; *R. napoensis* Li, Liu, Yu, & Sun, 2022; *R. orlovi* Ziegler & Köhler, 2001; *R. rhodopus* Liu & Hu, 1960; *R. translineatus* Wu, 1977; and *R. tuberculatus* (Anderson, 1871) (Che et al. 2020; Naveen et al. 2023; AmphibiaChina 2024). Recent studies suggest that the overall species richness of *Rhacophorus* is underestimated (Kropachev et al. 2022; Li et al. 2022). The reevaluations of the "widespread" species and the survey of unexplored areas are likely to reveal overlooked diversity.

During the recent herpetological surveys in Dulongjiang Village, northern Gaoligong Mountains, Yunnan Province, China, we collected unidentified specimens of a *Rhacophorus* population, which differed from other congeneric members by both morphological and molecular characteristics. As a result, we herein describe it as a new species.

Materials and methods

Sampling

During a field survey at Dulongjiang Village, Gongshan County, Nujiang Prefecture, Yunnan Province, China, in February 2022 (Fig. 1), two adult specimens (one male and one female) of *Rhacophorus* were collected and photographed. Sex was determined by the presence of internal vocal sac openings and the presence of eggs in the abdomen, as observed via external inspection. The specimens were then euthanized, fixed in 10% formalin, and subsequently stored in 75% ethanol for 24 hours. Liver tissues were taken and preserved in 95% alcohol. The procedures for DNA tissue sampling and specimen fixation follow the protocols detailed in Chen et al. (2021). Voucher specimens were deposited at Anhui Normal University (ANU), China. The protocols (No. SYDW-20130814-71) of the Animal Care and Ethics Committee were followed for the proper treatment of animals.

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from liver tissue stored in 95% ethanol using the standard phenol-chloroform extraction protocol (Sambrook et al. 1989). The partial sequences of the mitochondrial 16S rRNA gene from the new samples were amplified and sequenced using the primers in Yu et al. (2019). PCR amplification was performed in a 25 µl reaction volume with the following cycling conditions: initial denaturation step at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 55°C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR products were purified using the Gel Extraction Mini Kit (Watson BioTechnologies, Shanghai, China) and then sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Newly generated sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

Newly obtained nucleotide sequences were first assembled and edited using DNASTAR LASERGENE 7.1. To obtain the phylogenetic relationships among *Rhacophorus*, homologous sequences of all *Rhacophorus* species available in the NCBI GenBank were downloaded (Table 1). Our final dataset included 37 described species of *Rhacophorus*. In cases of geographically widespread species, multiple samples from different localities were included. *Zhangixalus burmanus* and *Z. wui* were chosen as outgroups based on previous phylogenetic studies (Jiang et al. 2019). New sequences incorporated with the data retrieved from GenBank were aligned using MUSCLE 3.8 (Edgar 2004) and then inspected by eye for accuracy and trimmed to minimize missing characters in MEGA11 (Tamura et al. 2021). The aligned length of the complete 16S rRNA data matrix was 969 bps.

The phylogenetic reconstruction was performed using Bayesian (BI) analyses and maximum likelihood

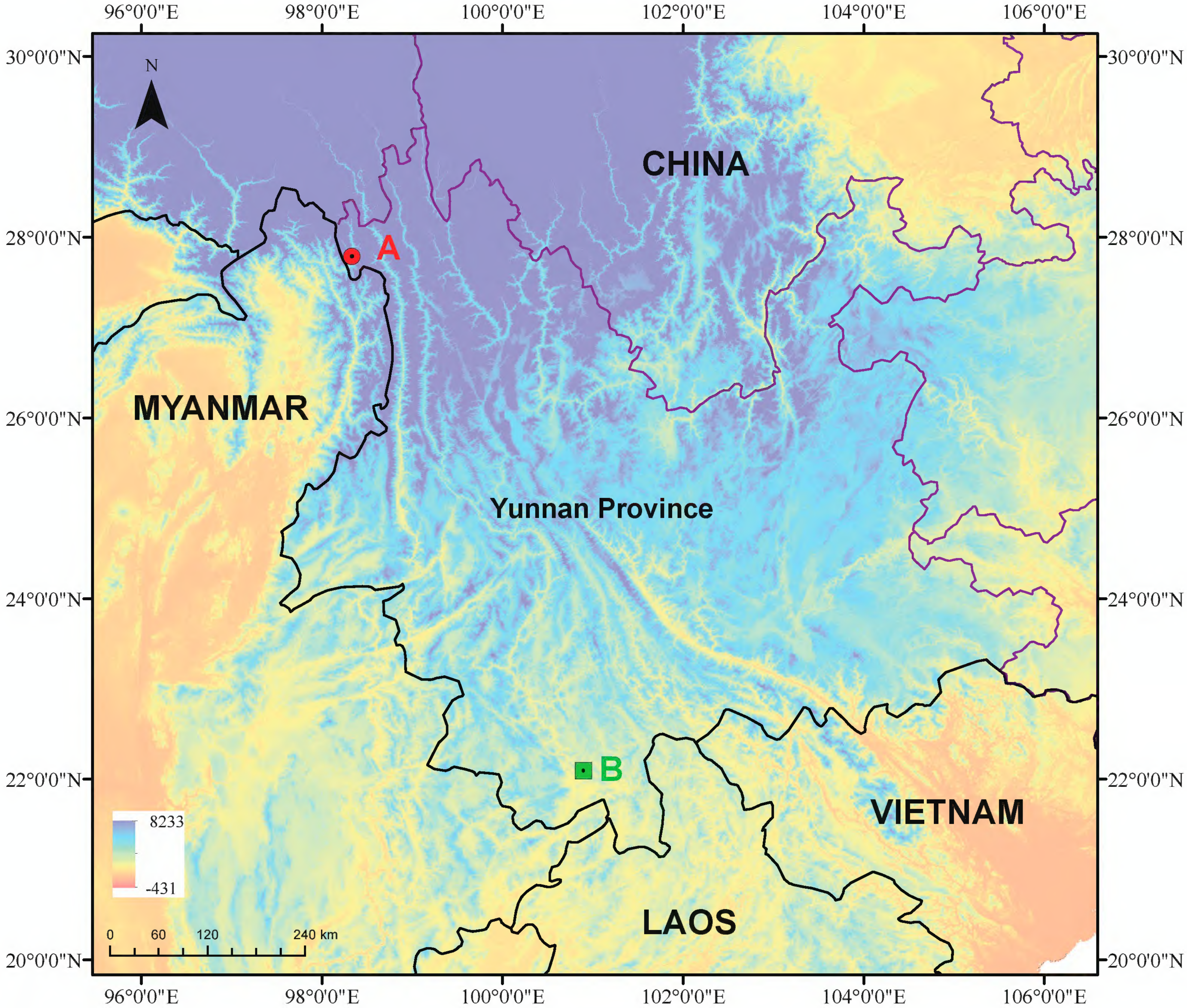

Figure 1. Map showing the collection site and type locality of *Rhacophorus dulongensis* sp. nov. (**A**; red circle) and *R. rhodopus* (**B**; green square).

(ML) methods for the 16S rRNA gene. The best-fit model of evolution for 16S rRNA was determined using the Bayesian Information Criterion (BIC) by jModelTest 2.1.7 (Darriba et al. 2012). The BI analyses were conducted in MrBayes 3.2 (Ronquist and Huelsenbeck 2003) with 30 million generations and sampled every 1000 generations. Convergence was assessed in Tracer 1.5 (Rambaut and Drummond 2009) based on having an average standard deviation of split frequencies less than 0.01 and ESS values greater than 200. We excluded the first 25% of trees before the log-likelihood scores stabilized as burn-in. The ML analyses were performed using RAxML-HPC BlackBox 8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2010). The analyses used the proportion of invariable sites estimated from the data and 1000 bootstrap pseudoreplicates under the GTR+gamma model. Nodes in the trees were considered well-supported when ML bootstrap support (BS) was ≥ 75% and Bayesian posterior probabilities (PP) were ≥ 0.95. Uncorrected pairwise distances (*p*-distances) between species were calculated in MEGA 11 (Tamura et al. 2021).

Morphology and morphometrics

Measurements were recorded to the nearest 0.1 mm with digital calipers by Renda Ai following Fei et al. (2009) and Li et al. (2022). The measurements taken were as follows: SVL (snout-vent length, the length from the tip of snout to vent); HL (head length, the length from the tip of snout to the posterior edge of the mandibular joint); HW (head width, the maximum distance between two sides of the head); SL (snout length, the length from the tip of snout to anterior border of eye); INS (internasal space, the distance between the inner edges of the left and right nostrils); IOS (interorbital space, the narrowest distance between the medial edges of the left and right upper eyelids); UEW (width of upper eyelid, the maximum width of upper eyelid); ED (diameter of eye, the diameter of the eye parallel to the body axis); TD (diameter of tympanum, the maximum diameter of tympanum); DNE (distance from nostril to eye, the length from the anterior border of the eye to the inner edge of the ipsilateral nostril); SN (distance from snout to nostril, the length from the tip of snout to the inner edge of the

Table 1. Information for samples used in the molecular phylogenetic analyses in this study. New sequences generated for the present study are PP574166–PP574167.

	Species	Voucher	Locality	GenBank No.
Ingroup				
1	*R. dulongjiang* sp. nov.	ANU010645	Dulongjiang Village, Yunnan, China	PP574166
2	*R. dulongjiang* sp. nov.	ANU010646	Dulongjiang Village, Yunnan, China	PP574167
3	*R. annamensis*	KIZ1196	Binh Thuan, Vietnam	JX219446
4	*R. baluensis*	FM235958	Tambunan District, Sabah, Malaysia	KC961089
5	*R. barisani*	UTAA-61235	Gunung Dempo, Provinsi Sumatera Selatan, Indonesia	KC701714
6	*R. bengkuluensis*	UTAA-62770	Kabupaten Lampung Barat, Lampung, Sumatra	KM212948
7	*R. bipunctatus*	CAS229913	Au Yin Gacamp, Kachin State, Myanmar	JX219445
8	*R. bipunctatus*	PUCZM/IX/SL612	Mizoram, India	MH087076
9	*R. borneensis*	BORN:22410	Maliau Basin, Sabah, Malaysia	AB781693
10	*R. calcadensis*	SDB.2011.291	Kadalar, Idukki, Kerala	KC571276
11	*R. calcaneus*	KIZ528	Bi Doup National Park, Lam Dong, Vietnam	JX219450
12	*R. catamitus*	ENS7657	Sumatra, Indonesia	JF748387
13	*R. edentulus*	MZB:Amp:30875	Gunung Katopasa, Sulawesi, Indonesia	MH751448
14	*R. exechopygus*	VNMN:4107	Gia Lai, Vietnam	LC010585
15	*R. gauni*	FMNH273928	Bintulu Division, Sarawak, Malaysia	JX219456
16	*R. georgii*	MZB:Amp:23395	Suaka Marga Satwa Nantu, Sulawesi, Indonesia	MH751453
17	*R. helenae*	NAP03164	Ba Ria-Vung Tau, Vietnam	KX139175
18	*R. hoabinhensis*	IEBRA.2016.18	Hoa Binh, Vietnam	LC331096
19	*R. indonesiensis*	MZB:Amp:23619	Birun Village, Jambi Province, Indonesia	AB983367
20	*R. kio*	KUHE:55167	Than Hoa, Vietnam	AB781697
21	*R. laoshan*	1705014	Guangxi, China	MW149528
22	*R. lateralis*	RBRL050709-35,36,37	Mudigere, India	AB530548
23	*R. malabaricus*	Release	Madikeri, India	AB530549
24	*R. margaritifer*	ENS16162	Tilu, Indonesia	KX398889
25	*R. modestus*	ENS16853	Samosir, Sumatra, Indonesia	KX398904
26	*R. monticola*	RMB1236	Mt. Lompo Batang, Sulawesi , Indonesia	AY326060
27	*R. napoensis*	GXNUYU000171	Napo, Guangxi, China	ON217796
28	*R. nigropalmatus*	Rao081204	Malaysia	JX219437
29	*R. norhayatii*	KUHEUNL	Endau Rompin, Johor, Malaysia	AB728191
30	*R. orlovi*	LJTR44	Maguan, Yunan, China	KC465840
31	*R. pardalis*	FMNH273245	Bintulu Division, Sarawak, Malaysia	JX219453
32	*R. poecilonotus*	ENS16480	Sibayak, Sumatra, Indonesia	KX398920
33	*R. pseudomalabaricus*	SDB.2011.1010	Kadalar, Kerala, India	KC593855
34	*R. reinwardtii*	ENS16179	Patuha, Java, Indonesia	KY886328
35	*R. rhodopus* (Lineage 1)	KIZYPX20553	Mengyang, Yunnan, China	MW133350
36	*R. rhodopus* (Lineage 2)	Loc08007018	Longchuan, Yunnan, China	JX219439
37	*R. rhodopus* (Lineage 2)	KIZ587	Longling, Yunnan, China	EF564577
38	*R. rhodopus* (Lineage 3)	L062456	Mêdog, Tibet, China	JX219442
39	*R. rhodopus* (Lineage 4)	SN030035	Hainan, China	EU215529
40	*R. rhodopus* (Lineage 4)	VNMN:4117	Gia Lai, Vietnam	LC010604
41	*R. rhodopus* (Lineage 5)	VNMN:4118	Bac Giang, Vietnam	LC010605
42	*R. robertingeri*	VNMN:4123	Gig Lai, Vietnam	LC010613
43	*R. rufipes*	FMNH272858	Bintulu Division, Sarawak, Malaysia	JX219455
44	*R. spelaeus*	IEBRA.2011.1	Khammouan, Laos	LC331095
45	*R. translineatus*	KIZ06648	Mêdog, Tibet, China	MW111521
46	*R. tuberculatus*	KIZ014154	Mêdog, Tibet, China	MW111522
Outgroup				
47	*Zhangixalus burmanus*	SCUM060614L	Mt. Gaoligong, Yunnan, China	EU215537
48	*Zhangixalus wui*	CIB097690	Lichuan, Hubei, China	JN688880

ipsilateral nostril); LAHL (length of lower arm and hand, the length from elbow joint to the tip of the third finger); HAL (hand length, from proximal end of outer palmar tubercle to tip of the third finger); TYE (distance from anterior margin of tympanum to posterior corner of eye); HLL (hindlimb length, measured as length of straightened hindlimb from groin to tip of fourth toe); THL (thigh length, the length from vent to knee); TIL (tibia length, the length from knee to ankle); TFL (length of the foot and tarsus, the length from the tibial tarsal joint to the tip of the fourth toe); FL (foot length, the length from the proximal end of the medial metatarsal process to the tip of the fourth toe). The description of webbing formula followed Guayasamin et al. (2006).

Results

Morphology

The two collected specimens were assigned to the genus *Rhacophorus* based on the following morphological characters: intercalary cartilage between terminal and penultimate phalanges of digits present, Y-shaped distal end of terminal phalanx, finger tips expanding into large disks bearing circum-marginal grooves, webbing exists between all fingers, skin not co-ossified with the skull, pupil horizontal, extensive dermal folds exist on the limbs, and dorsal color predominantly brown or green (Li et al. 2012; Jiang et al. 2019; AmphibiaChina 2024).

The samples of *Rhacophorus* collected in Dulongjiang Village, Gongshan County, Nujiang Prefecture, Yunnan Province were reliably differentiated from all known congeners based on the body size, head length, tibia length, snout and tongue shape, toe webbing formula and coloration, ventral skin texture and coloration, dorsal pattern and coloration, body macroglands, iris coloration, and pattern of markings on flanks, which supported the recognition of the new species.

Phylogenetics and genetic divergence

The BI and ML analyses resulted in essentially identical topologies (Fig. 2). Although the basal relationships within *Rhacophorus* were not well resolved, our analyses revealed two major clades, denoted A and B, within *Rhacophorus*. Clade A contained the majority of species of *Rhacophorus* and was widely distributed across Southeast Asia and southwestern China. Our new samples from Dulongjiang Village belong to Clade A, which strongly clustered into a lineage (BI = 1.00, ML = 100; Fig. 2) and clustered with *R. tuberculatus*, *R. orlovi*, and *R. spelaeus* from the eastern Himalayas, southwestern China, and northern Indochina with strong support (BI = 1.00, ML = 100; Fig. 2).

The putative new species from Dulongjiang Village showed obvious genetic divergence from other congeners. When compared with closely related recognized congeners, the minimum uncorrected genetic distance was 4.8% between the clade from Dulongjiang Village and *R. tuberculatus* (Table 2). These levels of pairwise divergence of the 16S rRNA gene obviously exceeded the accepted threshold of species-level genetic divergence in anurans (3.0%; Vences et al. 2005) and are greater than some known interspecific distances for *Rhacophorus* (Table 2). In addition, the analysis showed that *R. rhodopus* is not monophyletic and is a composite of five phylogenetically distinct lineages (Fig. 2). The sequence divergence in the 16S rRNA gene among the five lineages of *R. rhodopus* is 4.7–9.1% (Table 2). The samples of *R. rhodopus* from Bac Giang, Vietnam (Lineage 5; GenBank accession numbers: LC010605; Fig. 2) strongly clustered with *R. napoensis* with only 0.2% sequence divergence.

Taken together, our results indicated that the observed molecular divergence of the newly discovered population of *Rhacophorus* from Dulongjiang Village is concordant with stable differences in diagnostic morphological character, which distinguish it from all known congeners (see Comparisons). Thus, upon combining morphological and molecular lines of evidence, we herein describe this distinct lineage of *Rhacophorus* as a new species.

Taxonomic account

Rhacophorus dulongensis **Chen, Lee & Yuan, sp. nov.**
https://zoobank.org/976AF923-DDF9-4E9A-BF72-0B2141DF493C
Figs 3, 4

Type materials. ***Holotype.*** ANU010645, adult male, collected from Dulongjiang Village, Gongshan County, Nujiang Prefecture, Yunnan Province, China (27.7838°N, 98.3248°E, 1620 m a.s.l.; Fig. 1) on 15 February 2022, by Renda Ai. ***Paratypes.*** ANU010646,

Table 2. Sequence divergences based on uncorrected *p*-distances (%) of 16S rRNA between the new species and its close congeners of *Rhacophorus* used in this study.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*R. dulongensis* sp. nov.															
2	*R. rhodopus* (Lineage 1)	15.0														
3	*R. rhodopus* (Lineage 2)	11.6	4.8													
4	*R. rhodopus* (Lineage 3)	13.6	8.2	6.0												
5	*R. rhodopus* (Lineage 4)	12.4	7.4	9.1	6.8											
6	*R. rhodopus* (Lineage 5)	10.5	8.1	7.7	4.7	6.5										
7	*R. napoensis*	12.9	7.7	6.6	7.1	6.8	0.2									
8	*R. tuberculatus*	4.8	13.8	10.7	12.0	11.0	9.3	12.0								
9	*R. orlovi*	7.5	14.1	11.8	13.3	13.5	11.2	12.8	7.7							
10	*R. spelaeus*	8.6	12.8	13.9	11.6	13.3	11.6	11.6	8.8	3.7						
11	*R. bipunctatus*	14.1	10.2	6.6	8.5	8.4	5.8	7.7	13.0	14.0	11.3					
12	*R. poecilonotus*	10.2	8.9	7.6	7.2	10.2	8.8	7.8	9.1	10.0	10.9	8.0				
13	*R. barisani*	9.5	8.4	7.5	6.7	9.4	8.4	7.3	8.8	9.3	10.2	7.5	2.2			
14	*R. bengkuluensis*	12.3	10.2	9.2	9.1	11.2	10.7	9.3	11.2	10.4	10.2	9.8	8.3	7.7		
15	*R. margaritifer*	11.8	9.7	9.1	8.3	10.7	10.7	9.3	11.0	11.2	10.9	10.1	6.4	6.6	4.3	

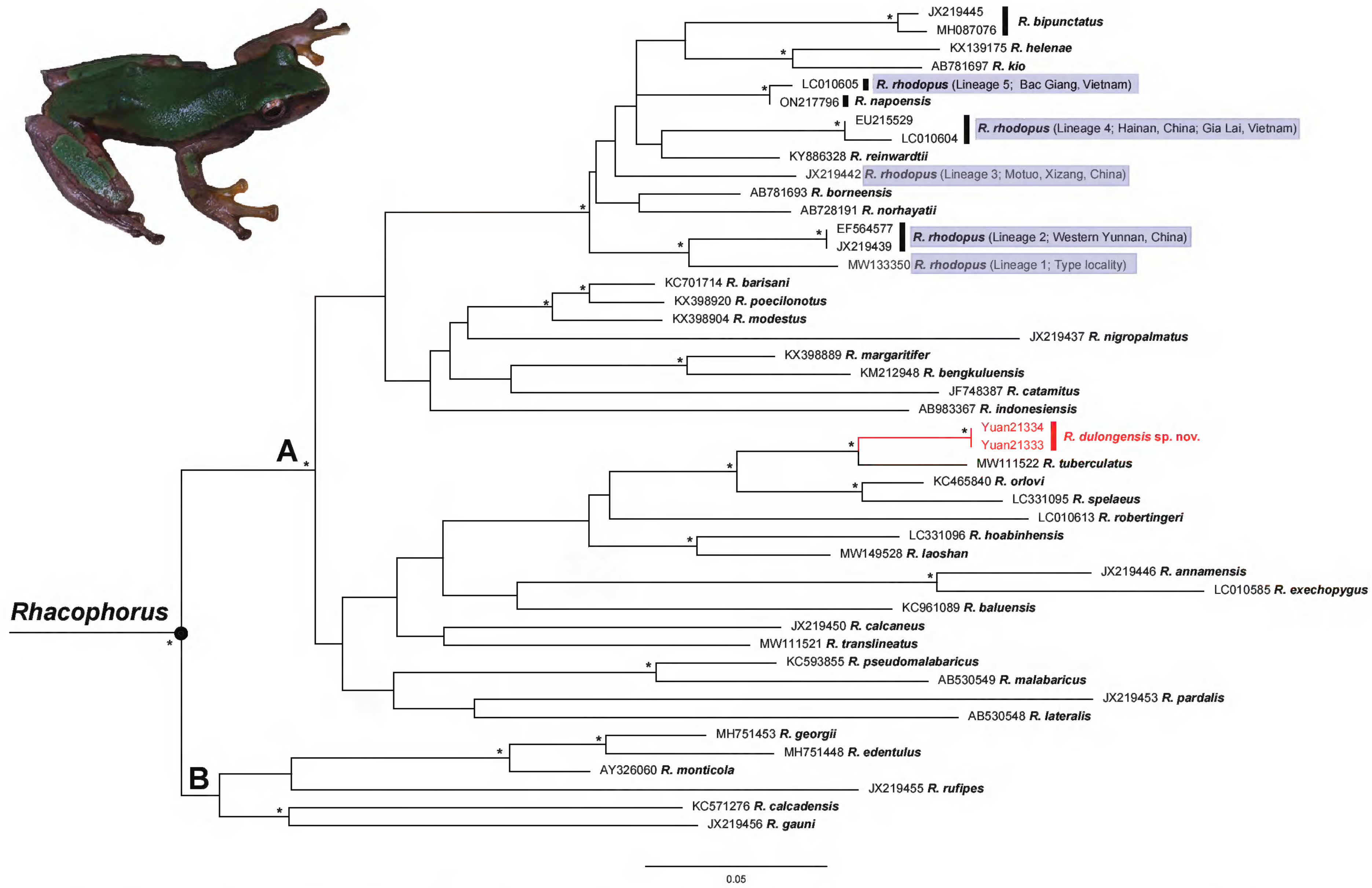

Figure 2. Phylogram of *Rhacophorus* inferred from a 16S rRNA mtDNA gene fragment. "*" denotes high support by bootstrap support values (BS > 75%) and Bayesian posterior probabilities (BPP > 0.95); nodes without any numbers or symbols represent low support values (BS < 75% and BPP < 0.95). Outgroup taxa are not shown. The inserted photo (acknowledgment to Renda Ai) shows *Rhacophorus dulongensis* sp. nov. Scale bars represent the number of substitutions per site. The new samples for the present study are indicated by the red font. *R. rhodopus* is framed by the purple shade.

adult female, collected at the same time as the holotype from the type locality by Renda Ai.

Etymology. The specific epithet "dulongensis" is given as a noun in apposition and refers to the name of the Dulongjiang Village, where the new species occurs. We suggest the English common name "Dulongjiang tree frog" and the Chinese common name "独龙江树蛙" (dú lóng jiāng shù wā).

Diagnosis. *Rhacophorus dulongensis* sp. nov. can be distinguished from its congeners by the following combination of morphological characters: (1) body size small (SVL 31.7 mm in male; 35.3 mm in female); (2) head length longer than head width; (3) tibia length shorter than half of snout-vent length; (4) third finger disk smaller in diameter than tympanum; (5) snout pointed without a distinct bulge; (6) the tibiotarsal articulation reaches the eye when hindlimb is stretched along the side of the body; (7) dorsal surface of body uniformly green, and dorsal surface of limbs brown with irregular green patches; (8) belly mostly yellowish, rough, and granular (9) large black warts present on ventral surface of thigh near vent; (10) webs between toes red and webbing formula on toes: $I1^{0}\text{-}1^{2/3}II1^{-}\text{-}2^{1/2}III1^{+}\text{-}2^{1/3}IV2^{1/3}\text{-}1^{1/3}V$; (11) black spots at axillary region absent; (12) vomerine teeth weakly developed; (13) iris darkgoldenrod; (14) maxillary teeth distinct; (15) tongue notably notched posteriorly.

Description of holotype. Adult male, body size small (SVL 31.7 mm); head length (HL 9.8 mm) longer than head width (HW 8.7 mm); snout pointed, protruding from the margin of the lower jaw, longer (SL 4.6 mm) than diameter of eye (ED 3.5 mm); canthus rostralis distinct; loreal region oblique; nostril small, closer to tip of snout than to eye; interorbital space (IOS 2.8 mm) longer than internasal space (INS 2.5 mm) and width of upper eyelid (UEW 2.1 mm); pupil horizontal and iris darkgoldenrod; pineal ocellus absent; tympanum rounded and distinct, diameter of tympanum (TD 1.7 mm) shorter than half of eye diameter (ED 3.5 mm), internasal space (INS 2.5 mm) and interorbital space (IOS 2.8 mm); supratympanic fold distinct; maxillary teeth distinct; vomerine teeth weak; internal single subgular vocal sac; vocal sac openings small, slit-like; tongue heart-shaped, attached anteriorly, with distinct notch at posterior end; choanae oval (Table 3).

Forelimbs thin, slender and not very long; length of lower arm and hand (LAHL 13.4 mm) shorter than half snout-vent length (SVL 31.7 mm); relative length of fingers: III>IV>II>I; tips of fingers expanded into discs; finger webbing formula: $I2^{+}\text{-}2^{1/3}II1^{+}\text{-}2^{2/3}III2^{1/3}\text{-}1^{2/3}IV$; subarticular tubercles distinct, blunt and round; third finger disk shorter than diameter of tympanum (TD 1.7 mm); supernumerary tubercles below the base of finger present;

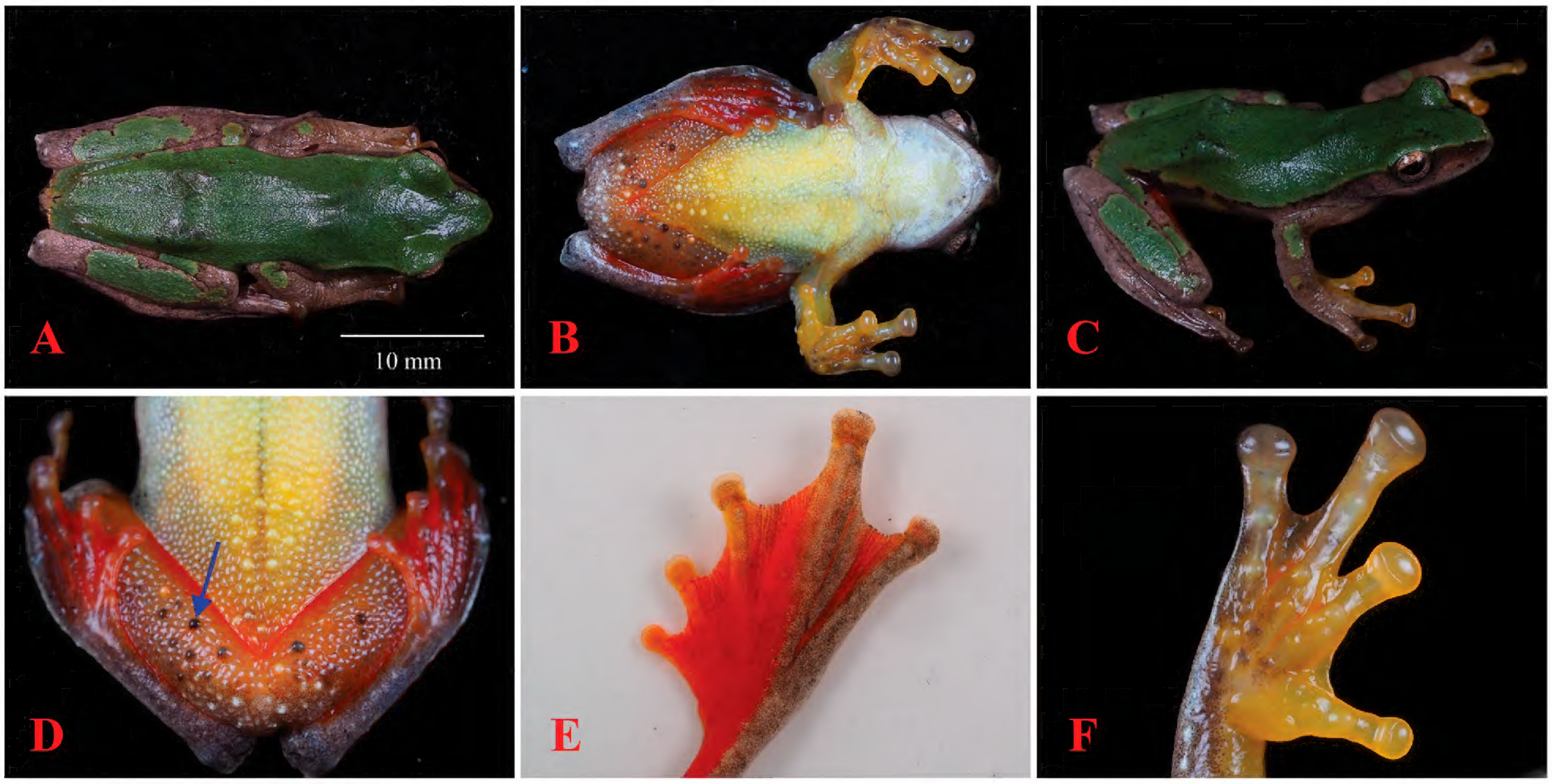

Figure 3. Holotype of *Rhacophorus dulongensis* sp. nov. (ANU010645) in life. **A.** Dorsal view; **B.** Ventral view; **C.** Dorso-lateral view; **D.** Ventral surface of the thigh and the cloacal region; **E.** Plantar view of the left foot; **F.** Thenar view of the right hand. The blue arrow in **D** points to large black warts. Photos by Renda Ai.

Table 3. Measurements of the type series of *Rhacophorus dulongensis* sp. nov. and *R. turpes* (all measurements are in mm). N/A indicates that data are lacking for that morphological index. Abbreviations are defined in the text.

Species	*R. dulongensis* **sp. nov.**		*R. turpes*	
	Holotype	Paratype	Syntype	Paratype
Voucher Nos.	ANU010645	ANU010646	BMNH1940.6.1.30	BMNH1974.832
Sex	Male	Female	Female	Female
SVL	31.7	35.3	37.4	35.8
HL	9.8	10.5	12.2	10.8
HW	8.7	8.8	9.7	9.8
SL	4.6	4.3	4.9	5.4
INS	2.5	2.3	2.8	3.1
IOS	2.8	3.2	4.3	4.1
UEW	2.1	2.3	2.4	2.0
ED	3.5	3.4	3.5	3.0
TD	1.7	1.9	2.4	2.8
SN	1.2	1.5	N/A	N/A
LAHL	13.4	13.8	N/A	N/A
HAL	7.9	8.5	9.2	8.6
TYE	0.6	0.8	1.1	1.2
HLL	49.1	48.9	N/A	N/A
THL	15.2	14	14.9	16.2
TIL	14.6	15.1	16.9	18.3
TFL	19.3	19.8	N/A	N/A
FL	13.1	12.6	10.8	10.4
DNE	2.7	3.2	2.7	3.3

nuptial pads absent; inner metacarpal tubercle distinct, large and oval (Fig. 3; Table 3).

Hindlimbs slender, relatively long, tibia length (TIL 14.6 mm) shorter than half of snout-vent length (SVL 31.7 mm) and thigh length (THL 15.2 mm); tibiotarsal articulation reaches the eye when hindlimb is stretched along the side of the body; heels overlapping when held at right angles to the body; relative length of toes:

Figure 4. Type series of *Rhacophorus dulongensis* sp. nov. in preservation. **A.** ANU010645; **B.** ANU010645; **A1.** Dorsal view; **A2.** Ventral view; **B1.** Dorsal view; **B2.** Ventral view. The blue arrows in **A2** and **B2** point to large black warts. Photos by Renda Ai.

IV>V>III>II>I; tips of toes expanded into discs; subarticular tubercles on all toes round, distinct, and protuberant; entire web between toes; toes webbing formula: $I1^{0}$-$1^{2/3}II1^{-}$-$2^{1/2}III1^{+}$-$2^{1/3}IV2^{1/3}$-$1^{1/3}V$; inner metatarsal tubercle small; outer metatarsal tubercle absent (Fig. 3; Table 3).

The dorsal surface of body smooth and uniformly green; dorsal surface of limbs brown with irregular green patches; the skin of throat, chest, and ventral surface of tibia, foot, and tarsus smooth; black dusting present on the margin of the throat; the belly mostly yellowish and rough; large black warts present on ventral surface of thigh near vent; dermal fringe along the outer sides of limbs indistinct; webs between toes red; black spots at axillary region absent.

Color of holotype in preservative. Dorsal color darkened; ventral surface faded to creamy white; brown dusting present on ventral surfaces of throat. The patterns of dark spots and markings all over the body are the same as in life. Large black warts on the ventral surface of the thigh near the vent are more distinct (Figs 3, 4).

Male secondary sexual characteristics. Nuptial pad and lineae masculinae were not observed.

Morphological variation. The paratype matches the overall characters of the holotype (Table 3; Fig. 4). Female larger than male (SVL 35.3 mm in one female and 31.7 mm in one male). The male has small slit-like vocal sac openings. Dusting pattern on the belly, chest, throat, and ventral surfaces of limbs varies individually. Female has more distinct brown dusting on the venter than male (Figs 3, 4).

Distribution and ecology. *Rhacophorus dulongensis* sp. nov. is presently known only from its type locality, near Dulongjiang Village, Gongshan County, Nujiang Prefecture, Yunnan Province, China. Both individuals of the new species were found inside bamboo internodes along rocky streams in well-preserved montane evergreen broadleaf forest (Fig. 5). Vocal recordings and tadpoles of this new species were not collected.

Comparisons. *Rhacophorus dulongensis* sp. nov. is distinguishable from all other species of *Rhacophorus* by a combination of features of body size, head length, tibia length, snout and tongue shape, toe webbing formula and coloration, ventral skin texture and coloration, dorsal pattern and coloration, body macroglands, iris coloration, and pattern of markings on flanks (Tao et al. 2014; Fei et al. 2009; Li et al. 2012; Che et al. 2020; Li et al. 2022; Naveen et al. 2023).

In particular, *R. dulongensis* sp. nov. can be easily distinguished from its four morphologically and phylogenetically close congeners (*R. tuberculatus*, *R. orlovi*,

Figure 5. Habitat of *Rhacophorus dulongensis* sp. nov. at the type locality in Dulongjiang Village, Yunnan Province, China. **B, C.** show the native bamboo species, *Cephalostachyum virulentum*, where the new species inhabits. Photos by Ying-Chun Li.

R. spelaeus, and *R. turpes*; Figs 2, 6; Table 3). Specifically, it differs from *R. tuberculatus* by having green dorsum coloration in life (vs. uniform brown dorsum coloration), different webbing formula on toes (I1^{0}-1$^{2/3}$II1^{-}-2$^{1/2}$III1^{+}-2$^{1/3}$IV2$^{1/3}$-1$^{1/3}$V vs. I1$^{1/2}$-2$^{1/2}$II1$^{1/3}$-2$^{1/2}$III1^{+}-2$^{1/3}$IV2^{-}-1^{-}V), as well as the absence of a prominent calcar at tibio-tarsal articulation (vs. presence) and the absence of dark stripes on the hindlimb (vs. presence).

R. dulongensis sp. nov. differs from *R. orlovi* by having smaller head (HL/SVL = 0.30–0.31 vs. HL/SVL = 0.38–0.41), different webbing formula on toes (I1^{0}-1$^{2/3}$II1^{-}-2$^{1/2}$III1^{+}-2$^{1/3}$IV2$^{1/3}$-1$^{1/3}$V vs. I1^{0}-2^{0}II1^{0}-2^{0}III1^{0}-2^{0}IV2^{0}-1^{0}V), green dorsal coloration in life (vs. reddish brown), hindlimb without transverse stripes (vs. limbs with transverse stripes), as well as the presence of large black warts on the ventral surface of the thigh (vs. absence) and the absence of spotting on flanks (vs. presence).

R. dulongensis sp. nov. differs from *R. spelaeus* by having smaller body size in males (SVL 31.7 vs. 38.9–43.1 mm), different webbing formula on toes (I1^{0}-1$^{2/3}$II1^{-}-2$^{1/2}$III1^{+}-2$^{1/3}$IV2$^{1/3}$-1$^{1/3}$V vs. I0-1II0-1/2III1-0V1/2-1/2V), green dorsal coloration in life (vs. grey-brown coloration in life), ventral surface of belly yellowish (vs. light gray), as well as the presence of vomerine teeth (vs. absence).

Specifically, *R. dulongensis* sp. nov. differs from *R. turpes* by having hindlimbs without dark stripes (vs. hindlimbs with dark stripes), by different webbing formula on toes (I1^{0}-1$^{2/3}$II1^{-}-2$^{1/2}$III1^{+}-2$^{1/3}$IV2$^{1/3}$-1$^{1/3}$V vs. I1$^{1/3}$-1$^{1/2}$II1$^{1/2}$-1$^{2/3}$III1^{0}-1$^{1/2}$IV2^{0}-1^{0}V), vomerine teeth weak and small (vs. vomerine teeth distinct and large), dermal projection on the heel poorly developed (vs. well-developed), having a relatively longer foot length (FL/SVL ratio 0.36–0.41 in *R. dulongensis* sp. nov. vs. 0.29 in *R. turpes*), numerous and large black warts on ventral surface of thigh (vs. few and small in *R. turpes*), as well as the absence of small black spots in male (vs. presence in *R. turpes*) (Fig. 6).

In addition, *R. dulongensis* sp. nov. further differs from *R. rhodopus*, the notoriously "widespread" species of *Rhacophorus* in China and Indochina, by head length longer than head width (vs. head length almost equal to head width), third finger disk shorter than diameter of tympanum (vs. third finger disk longer than diameter of tympanum), tibia length shorter than half of snout-vent length (vs. tibia length about half of snout-vent length), as well as the absence of the black spots at axillary region (vs. presence), the absence of dermal calcars on vent (vs. present), and the absence of transverse stripes on hindlimb (vs. presence).

Among the species that are geographically close to *R. dulongensis* sp. nov., it distinctly differs from *R. bipunctatus* by having distinct tympanum (vs. indistinct), head length longer than head width (vs. head length almost equal to head width), as well as the absence of dermal calcars on heels (vs. presence), and the absence of black spots at axillary region (vs. presence). *R. dulongensis* sp. nov. differs from *R. translineatus* by having smaller body size (SVL 31.7 mm in male, 35.3 mm in female vs. 49.4–54.1 mm in males, 61.5–65.2 mm in females), different webbing formula on toes (I1^{0}-1$^{2/3}$II1^{-}-2$^{1/2}$III1^{+}-2$^{1/3}$IV2$^{1/3}$-1$^{1/3}$V vs. I0-0II0-0III0-0-IV0-0V), as well as the absence of transverse dark brown lines on the back (vs. presence), and the absence of an appendage on the tip of snout (vs. presence). *R. dulongensis* sp. nov. differs from *R. subansiriensis* by having smaller body size in male (SVL 31.7 mm vs. 37.0–39.0 mm), head length longer than head width (vs. head length shorter than head width), as well as the absence of dark cross bands on limbs (vs. presence), and the absence of spots on flanks (vs. presence).

For the remaining congeners, *R. dulongensis* sp. nov. differs from *R. kio* by having smaller body size (SVL 31.7 mm in male, 35.3 mm in female vs. 58.0–79.1 mm in male, 82.6–88.9 mm in female), different webbing on toes (entirely webbed vs. fully webbed), red web (vs. web

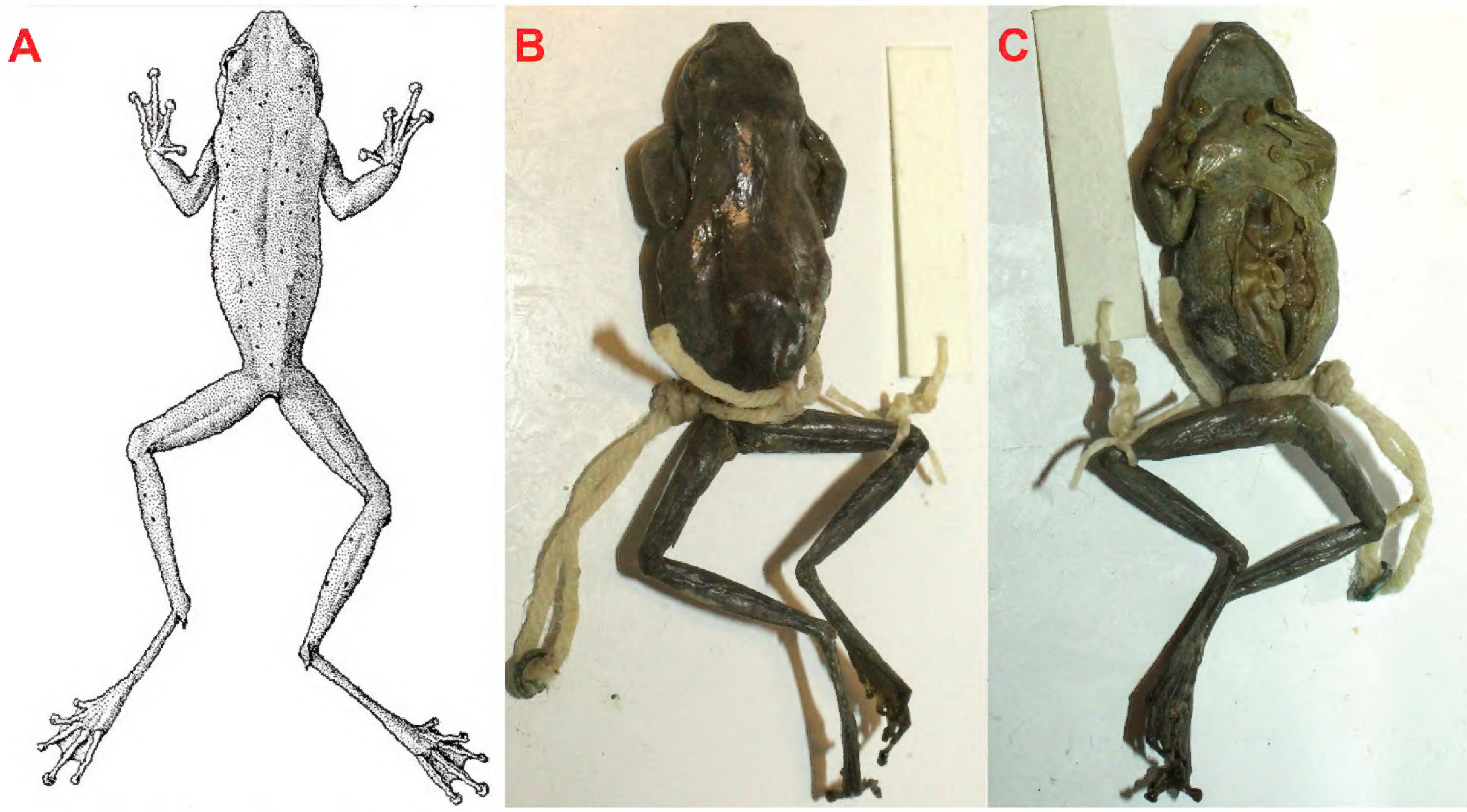

Figure 6. Type series of *Rhacophorus turpes*. **A.** The illustration of *R. turpes* in the original literature (Smith 1940); **B, C.** The syntype of *R. turpes* (BMNH1940.6.1.30) in the dorsal and ventral views, respectively (Photos by R. S. Naveen).

with black spot and orange yellow distal zone), as well as the absence of pointed dermal flap on heels (vs. presence), and the absence of black marking on flanks (vs. presence). *R. dulongensis* sp. nov. differs from *R. laoshan* by having fifth finger longer than third finger (vs. equal), different webbing formula on toes ($I1^{0}$-$1^{2/3}II1^{-}$-$2^{1/2}III1^{+}$-$2^{1/3}IV2^{1/3}$-$1^{1/3}V$ vs. I1-$2^{1/2}$II1-$2^{1/2}$III1-$2^{1/2}$IV2-1V), limbs without broad transverse stripes (vs. limbs with broad transverse stripes), and uniform green dorsum coloration in life (vs. chocolate brown dorsum coloration with wide dark cross-shaped mark). *R. dulongensis* sp. nov. differs from *R. napoensis* by having smaller body size in male (SVL 31.7 mm vs. 38.6–43.6 mm), head width shorter than head length (vs. head width longer than head length), the tibiotarsal articulation reaches the eye (vs. the tibiotarsal articulation reaches the snout), as well as the absence of a distinct bulge on the tip of snout (vs. presence), the absence of black spots at axillary region (vs. presence), and the absence of horizontal banding on dorsum and dorsal surface of limbs (vs. presence).

Lastly, *R. dulongensis* sp. nov. can be distinguished by its smaller body size in male (SVL 31.7 mm vs. 53.16 mm in *R. barisani*; 40.5–46.7 mm in *R. bengkuluensis*; 50.9 mm in *R. borneensis*; 60.0 mm in *R. helenae*; 49.9 mm in *R. larissae*; 64.7 mm in *R. norhayatiae*; 66.8 mm in *R. pseudomalabaricus*; over 58.0 mm in *R. reinwardtii*; 49.5–68.2 mm in *R. malabaricus*; 46.3 mm in *R. pardalis*; 45.9–46.4 mm in *R. exechopygus*); green dorsum coloration in life (vs. brown in *R. barisani*, *R. margaritifer* and *R. vanbanicus*; brown with darker cross bands in *R. bengkuluensis* and *R. catamitus*; yellowish grey with brown blotches in *R. hoabinhensis*; reddish brown with irregular dark brown blotches in *R. indonesiensis*; X-shaped blotch on the anterior part of the back in *R. monticola*; cream-colored in *R. marmoridorsum*; dark grey or brownish-grey in *R. calcadensis*; yellowish grey in *R. hoabinhensis*); head longer than head width (vs. head wider than long in *R. calcaneus*, *R. baluensis*, *R. trangdinhensis* and *R. viridimaculatus*); third finger disk shorter than diameter of tympanum (vs. third finger disk longer than diameter of tympanum in *R. calcaneus*, *R. annamensis*, *R. hoanglienensis*, *R. exechopygus*, *R. robertingeri* and *R. baluensis*); red webs between toes (vs. black webs in *R. borneensis*; proximally black and distally greenish webs in *R. helenae*; yellow webs in *R. edentulus*); tibiotarsal articulation reaches the eye (vs. tibiotarsal articulation reaches the tip of the snout in *R. nigropalmatus*; tibiotarsal articulation reaches beyond the tip of snout in *R. georgii*); absence of narrow dark cross-streaks on the limbs (vs. presence in *R. bifasciatus*); absence of a white streak on each side of body (vs. presence in *R. lateralis*); absence of a large triangular calcar heel (vs. presence in *R. robertingeri*).

Discussion

Our study further reveals the underestimation of species diversity and the taxonomic dispute in *Rhacophorus*. The discovery of *R. dulongensis* sp. nov. brings the total number of *Rhacophorus* species to 44 and the number of *Rhacophorus* known from China to 9. Besides, this study uncovers that the notoriously "widespread" species, *R. rhodopus*, is not monophyletic and is a composite of five phylogenetically distinct lineages (Fig. 2), as also revealed previously (e.g., Li et al. 2012; Li

et al. 2022). The divergence among the five lineages of *R. rhodopus* exceeds the threshold of species-level genetic divergence in anurans (3.0%; Vences et al. 2005) and is greater than some known interspecific distances for *Rhacophorus* (Table 2). Specifically, in addition to *R. rhodopus* from the type locality (Lineage 1), lineages 2–4 are likely to represent three new species (Fig. 2). Lineage 5 of *R. rhodopus* from northern Vietnam (Tao et al. 2014) is conspecific with *R. napoensis* with only 0.2% sequence divergence (Fig. 2; Table 2). *Rhacophorus napoensis* is currently only known from Baise City, Guangxi Province, China (Li et al. 2022; Frost 2024). Thus, our results revealed that *R. napoensis* is also found in Vietnam, making it a new country record for Vietnam, and we would formally correct the identity of LC010605 from Vietnam as *R. napoensis*. Detailed field investigations and more integrative studies, i.e., combining morphological, acoustic, and molecular data, are warranted to resolve these taxonomic disparities.

In addition, interestingly, both individuals of *R. dulongensis* sp. nov. were found inside the hollow internodes of the native bamboo species, *Cephalostachyum virulentum*. This behavior is a non-random event because its sister species, *R. tuberculatus*, has been reported to use bamboo internodes as breeding sites (Che et al. 2020). Moreover, this behavior has been reported in other genera of Rhacophoridae, such as *Raorchestes*. They usually enter the bamboo internodes through narrow openings and deposit eggs inside the bamboo. This elusive habit makes them easy to overlook (Seshadri and Bickford 2018; Che et al. 2020). However, the overharvesting of bamboo is threatening the survival of these bamboo nesting frogs (Seshadri et al. 2015). How this novel reproductive mode evolved is still unclear and needs further study.

Lastly, our study further highlights the lack of knowledge about the cryptic diversity in the Gaoligong Mountains. There was no previous record of the genus *Rhacophorus* from Dulongjiang Village (Yuan et al. 2022; AmphibiaChina 2024), where the new species was discovered in this study. Our finding and other recent discoveries (e.g., Yang et al. 2016; Chen et al. 2020; Liu et al. 2021; Wu et al. 2021) revealed that the increased expeditions to remote, previously unexplored areas largely contribute to the high discovery rate of new species in the Gaoligong Mountains. Besides, considering that the continuous forest habitats stretch across the international borders in this region, we expect that the new species found in this study may also occur in the adjacent regions of Myanmar. Further studies are needed to investigate the true extent of the occurrence of *R. dulongensis* sp. nov. Therefore, we propose that *R. dulongensis* sp. nov. be considered data deficient (DD) following the IUCN's Red List categories. With the increasing anthropogenic pressures and changing climatic conditions in the Gaoligong Mountains (Yang et al. 2019; Chen et al. 2020), focal biodiversity surveys and transboundary collaboration along the Chinese-Myanmar border are needed to detect more overlooked biodiversity and reinforce its value.

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